

**EXHIBIT A
ATTACHED TO
DECLARATION UNDER 37 CFR § 1.131
U.S. PATENT SERIAL NO. 09/614,631**

ECOLAB

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MICROBIOLOGICAL SERVICES REPORT

Rate of Kill of Eight New Formulations to Examine the Effects
of POOA, OA and NAS Against an *Aspergillus* sp. Isolate

M.S.R. #

SUBMITTED BY

John Hilgren, Food and Beverage Division

DATE SUBMITTED

REPORTED BY

COMPLETION DATE

Ex
A



Accredited by the Council for Antimicrobial Quality in Phase I of its Laboratory Accreditation Program for LP compliance and data integrity

OBJECTIVE:

The objective of the analysis was to determine the rate of kill of eight new formulations when tested at various concentrations of POOA, OA and NAS against an *Aspergillus* sp. field isolate.

TEST METHOD:

Ecolab Microbiological Services SOP ; Rate of Kill Antimicrobial Efficacy

METHOD PARAMETERS:

Test Substance Name	Concentration	Pre-test pH
Formula A	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 3 ppm POOA, 10 ppm OA	3.71
Formula B	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 17 ppm POOA, 10 ppm OA	3.72
Formula C	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 17 ppm POOA, 91 ppm OA	3.66
Formula D	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 17 ppm POOA, 91 ppm OA, 116 ppm NAS	3.67
Formula E	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 17 ppm POOA, 10 ppm OA, 116 ppm NAS	3.71
Formula F	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 3 ppm POOA, 91 ppm OA	3.69
Formula G	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 3 ppm POOA, 10 ppm OA, 116 ppm NAS	3.72
Formula H	80 ppm POAA, 16 ppm H ₂ O ₂ , 133 ppm Acetic Acid, 3 ppm POOA, 91 ppm OA, 116 ppm NAS	3.68

Diluent: Sterile Distilled Water

Test System: *Aspergillus* sp. (Gilroy Foods Isolate)

Test Temperature: 25°C

Exposure Times: 10, 20, 30 and 40 minutes

Neutralizer: 1% Sodium Thiosulfate

Subculture Medium: Sabouraud Dextrose Agar

Incubation: 26°C for 48 hours

RESULTS:

Test Date:
Date Results Read:

Inoculum Numbers (CFU/mL)

Test System	A	B	C	Average
<i>Aspergillus</i> sp. (Gilroy Foods Isolate)	8 x 10 ⁵	9 x 10 ⁵	8 x 10 ⁵	8.3 x 10 ⁵

Aspergillus sp.

Test Substance	Post-test pH	Exposure Time	Survivors (CFU/mL)	Average Survivors (CFU/mL)	Log Reduction	Percent Reduction
Formula A	4.02	10 minutes	19, 26 x 10 ⁵	2.2 x 10 ⁶	None	None
		20 minutes	11, 13 x 10 ⁵	1.2 x 10 ⁶	None	None
		30 minutes	8, 12 x 10 ⁵	1.0 x 10 ⁶	None	None
		40 minutes	7, 6 x 10 ⁵	6.5 x 10 ⁵	0.11	21.687
Formula B	3.74	10 minutes	39 x 10 ³ , <10	2.0 x 10 ⁴	1.54	97.590
		20 minutes	8, 32 x 10 ³	2.0 x 10 ⁴	1.54	97.590
		30 minutes	60 x 10 ¹ , 6 x 10 ³	3.3 x 10 ³	2.40	99.602
		40 minutes	28, 28 x 10 ³	2.8 x 10 ⁴	1.47	96.626
Formula C	3.69	10 minutes	11, 40 x 10 ³	2.6 x 10 ⁴	1.50	96.867
		20 minutes	3, 3 x 10 ³	3.0 x 10 ³	2.44	99.638
		30 minutes	8 x 10 ¹ , 3 x 10 ³	1.5 x 10 ³	2.74	99.819
		40 minutes	2 x 10 ¹ , 1 x 10 ³	5.1 x 10 ²	3.21	99.938
Formula D	3.71	10 minutes	32, 27 x 10 ³	3.0 x 10 ⁴	2.44	96.386
		20 minutes	52 x 10 ¹ , 5 x 10 ³	2.8 x 10 ³	2.47	99.663
		30 minutes	20 x 10 ¹ , 2 x 10 ³	1.1 x 10 ³	2.88	99.867
		40 minutes	1 x 10 ³ , 12 x 10 ¹	5.6 x 10 ²	3.17	99.932
Formula E	3.74	10 minutes	54, 66 x 10 ³	6.0 x 10 ⁴	1.14	92.771
		20 minutes	21, 42 x 10 ³	3.2 x 10 ⁴	1.41	96.144
		30 minutes	59 x 10 ¹ , 15 x 10 ³	7.8 x 10 ³	2.03	99.060
		40 minutes	12 x 10 ³ , 49 x 10 ¹	6.2 x 10 ³	2.13	99.253
Formula F	3.71	10 minutes	7, 17 x 10 ⁵	1.2 x 10 ⁶	None	None
		20 minutes	9, 10 x 10 ⁵	9.5 x 10 ⁵	None	None
		30 minutes	67, 68 x 10 ⁴	6.8 x 10 ⁴	1.09	91.807
		40 minutes	79, 68 x 10 ³	7.4 x 10 ⁴	1.05	91.084
Formula G	3.77	10 minutes	32, 16 x 10 ⁵	2.4 x 10 ⁶	None	None
		20 minutes	11, 14 x 10 ⁵	1.3 x 10 ⁶	None	None
		30 minutes	11, 11 x 10 ⁵	1.1 x 10 ⁶	None	None
		40 minutes	9, 17 x 10 ⁵	1.3 x 10 ⁶	None	None
Formula H	3.70	10 minutes	12, 20 x 10 ⁵	1.6 x 10 ⁶	None	None
		20 minutes	8, 14 x 10 ⁵	1.1 x 10 ⁶	None	None
		30 minutes	70, 109 x 10 ³	9.0 x 10 ⁴	0.96	89.157
		40 minutes	55, 79 x 10 ³	6.7 x 10 ⁴	1.09	91.928

CONCLUSIONS:

Formulas C and D were the most effective, achieving a >99.9% reduction of *Aspergillus* sp. after a 40 minute exposure time. The surfactant (NAS) did not appear to be a significant contributing factor in increased efficacy but the peroxyoctanoic acid (POOA) and the octanoic acid (OA) appeared to be the contributing factors in increased efficacy.

Tested and Certified by:

Date